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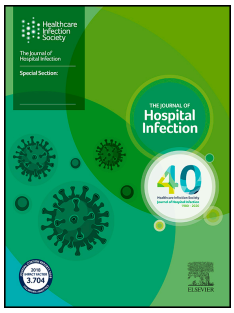

Prevalence and significance of bacterial contamination of autologous stem cell products

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Prevalence and significance of bacterial contamination of autologous stem cell products

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Ethical approval: this is a quality control study, whose purpose is to evaluate the effectiveness and efficiency of a practice that has been introduced and is already applied and compare this with the evidence-based standard that has already been published. According to the Swiss Ethics Committees on research involving humans (Swissethics), no ethical approval is required.

Keywords: contamination, autologous stem cell products, hematopoietic stem and progenitor cell products

Summary

There is limited and conflicting information on the prevalence of the contamination of hematopoietic stem and progenitor cell products (HPCPs) and their optimal management remains unclear. We reviewed the microbial surveillance data of HPCPs collected between 01/2002 and 12/2019 for autologous transplantation at our institution to determine the prevalence of microbial contamination and the potential infectious complications among recipients. Among 3935 HPCPs, 25 (0.6%) were contaminated. Ultimately, 22 patients received contaminated grafts, with a preemptive antimicrobial therapy initiated in 6/22. None developed subsequent infectious complications. Our data suggest that microbial contamination of autologous HPCPs and associated adverse outcomes are rare.

Introduction

Autologous hematopoietic stem cell transplantation following high-dose chemotherapy (ASCT/HDCT) is an effective option of consolidation of therapy in patients with multiple myeloma, lymphomas, and acute myeloid leukemia, and is also used in patients with poor-risk germ line tumors. Microbial quality control of hematopoietic stem and progenitor cell products (HPCPs) is a critical step in the transplant procedure. Previous studies assessing this issue among recipients of autologous or allogeneic grafts, reported a prevalence of contamination varying between 0.3% and 26.4%, predominantly involving species that are part of the normal skin flora [1-5]. Importantly, the management of a patient in whom contaminated HPCPs were infused is not standardized across studies and a variety of strategies have been proposed, including a preemptive antibiotic therapy in each recipient [1, 6, 7] or an adjusted antibiotic therapy in those cases where febrile neutropenia develops [3, 8]. Overall, major infectious complications such as subsequent bloodstream infection (BSI) appear to be rare [2, 3, 6, 9]. The objectives of this study were to determine the prevalence of microbial contamination in a large cohort of autologous stem cell recipients and describe management and outcome in those recipients given contaminated products.

Material and methods

At Bern University Hospital around 150 to 200 autologous stem cell transplantations (ASCTs) are performed each year, mostly as part of the consolidation therapy in patients with hemato-oncologic malignancies , but also in patients with germ line tumors. The entire process (including e.g. care for patients, apheresis, manufacturing process, cryopreservation, thawing, transplantation) is in accordance with current JACIE-standards (https://www.ebmt.org/about-jacie-standards). Our institution is continuously certified since 2003. Internal and external audits are performed in accordance with JACIE-specifications. At our institution, autologous HPCPs are systematically cultivated for quality control purposes. Usually, peripheral blood progenitor cells (PBPCs) are collected by a standard apheresis procedure through peripheral venipuncture or via central venous catheter. In rare situations, *e.g.* in case of insufficient peripheral stem cell mobilization, bone marrow (BM) is harvested from the posterior iliac crest by sterile technique as an alternative. Microbiological testing is routinely performed via bacterial and fungal culture at the time of apheresis or marrow harvesting, before cryopreservation and/or before infusion after thawing of cryopreserved products. The inoculum consists of 2-3 ml of HPCP. Cultures are performed following standardized procedures, and pathogens are identified and tested for antimicrobial susceptibility using CLSI (https://clsi.org) standards.

If microbiological growth is detected, the responsible hemato-oncologist is informed and the need for specific systemic preemptive antibiotic therapy before the infusion of the HPCP is evaluated on an individual basis together with an infectious disease specialist. In case of febrile neutropenia following the transplantation or at any sign suggestive of BSI two sets of blood cultures are drawn and a broad-spectrum antimicrobial therapy (*i.e.,* beta-lactam with or without vancomycin and/or metronidazole according to the possible focus) is initiated immediately. If, in the case of BSI, the same microorganism with identical resistance pattern is identified in both HPCP and blood cultures, the BSI is considered transplant-related. Since 2009, all patients admitted for autologous stem cell transplantation receive antimicrobial prophylaxis with trimethoprim/sulfamethoxazole (TMP-SMX, one tablet DS 3 times weekly), valacyclovir and fluconazole. No further systemic antibiotic prophylaxis (*e.g.*, ciprofloxacin) is routinely being administered to this patient population.

In this study, we retrospectively analyzed data of contaminated stem cell products from January 1, 2002 to December 31, 2019. Medical records of patients identified as having received contaminated HPCPs were

reviewed, and baseline characteristics, management and clinical outcomes were extracted. We recorded patients' demographic features, underlying neoplasia (hemato-oncologic entity vs solid tumors), cell source (BM or PBPCs), likely step of contamination, administration and type of antimicrobial prophylaxis and therapy, infusion-related symptoms, occurrence of fever and positive cultures following the infusion, infectious complications and in-hospital mortality. The patients were followed until the last oncological visit or right-censored at hospital discharge allowing analysis for long-term remission, relapse and mortality.

Results

During the study period, 3935 HPCPs were obtained from 1659 patients and cultured to rule out potential contamination. The overwhelming majority of them were PBPCs (3847, 98%). Twenty-five autologous stem cell products (24 PBPCs and 1 BM), mobilized from 24 patients, were found to be contaminated during the microbial testing process, resulting in a prevalence of 0.6% of contaminated products and a prevalence of 1.3% of involved patients. In 15 cases, the positive cultures were identified at the time of the apheresis procedure; in 10 cases, this happened later during the manufacturing process (before cryopreservation and/or before infusion after thawing of cryopreserved products, s. Table 1). Six out of 25 contaminations were polymicrobial (*i.e.*, >1 microorganism was observed), and overall a total of 34 microorganisms were cultured. Most of them (n=26, 76%) were considered typical skin contaminants, with coagulase-negative Staphylococci (CoNS) being the leading cause of contamination (n=23, 68%), followed by *Bacillus* spp (n=2, 6%) and *Cutibacterium acnes* (n=1, 3%). Of note, 87% of CoNS were oxacillin-sensitive, and based on 14 available sensibility assays, 100% were TMP-SMX sensitive. Other pathogens included S*treptococcus* spp (n=3, 9%), *Staphylococcus aureus* (n=1, 3%) and Gram-negative bacteria (n=4, 12%, s. Table 1). Of note, until the end of 2008 (when the prophylaxis with trimethoprim/sulfamethoxazole became a standard) we collected 1036 products, four of which were contaminated with eight microorganisms (7 ConS and one *St. aureus*). Since 2009, among 2899 collected products, 21 were contaminated with 26 microorganisms (16 CoNS, 3 streptococci, 2 *Bacillus* and one each for *Cutibacterium acnes*, *Acinetobacter* spp. *Veillonella* spp. *Ralstonia* spp. and one with not identified Gram-negative bacilli).

Contaminated grafts were infused in twenty-two patients (one patient died before the transplant; in another case the amount of stem cells was insufficient for the transplantation, and an additional collection procedure was initiated which turned out to be sterile). About two third of the recipients were male, and the median age was 51 years. Eighteen patients (82%) had a hemato-oncologic malignancy, predominantly multiple myeloma (n=9, 41%) or lymphoma subtypes (n=7, 32%), whereas two patients had relapsed germ line tumors (n=2, 8%). Sixteen patients (73%) were under antimicrobial prophylaxis with TMP-SMX, while two were under antibiotic therapy for other reasons (paronychia and fever of unknown origin, respectively) at the time of infusion. A preemptive antimicrobial therapy was initiated in six recipients of graft contaminated with CoNS (n=2), *Bacillus* spp, *Cutibacterium acnes, Ralstonia* spp, and a gram-negative bacterium not further specified. All but the

patient receiving the graft contaminated with *Bacillus* spp had previously been on antimicrobial prophylaxis with TMP-SMX.

Remarkably, none of the 22 recipients of contaminated products developed fever associated with the infusion of HPCPs (per definition on day 0). However, all but one of these recipients subsequently developed neutropenic fever (median interval: 6 days following the infusion of the autologous stem cells) and received a broad empiric antibiotic regimen after sampling of blood cultures. In none of the cases was the febrile episode found to be related to the graft contamination (*i.e.*, blood cultures remained negative, showed growth of another pathogen or revealed the same pathogen with a different resistance pattern, respectively). In all cases except one, the usual empiric therapy used at our institution to treat febrile neutropenia was adequate, based on susceptibility assays, for the treatment of the contaminant pathogen. One patient died because of candidemia during his post-transplant course. In the follow-up observation period (median duration: 129 weeks) seventeen patients were alive and in remission, two had experienced relapse of the underlying malignancy, and three had died (causes of death: hemato-oncologic malignancy, unknown, and the aforementioned candidemia, respectively).

Discussion

Microbial contamination of HPCPs is a potential threat for recipients of stem cell transplantation and may occur at any stage during this process [5, 6]. Routine culture of stem cell products to rule out contamination is generally considered a critical step in the transplant manufacturing procedure; however, relevant aspects of management of contaminated HPCPs differ across medical centers and research studies.

With almost 4,000 autologous HPCPs cultured at one center, to the best of our knowledge ours is among the largest studies on this matter published so far. Moreover, recent data on this topic are scant. Thus, these data are able to contribute to quality control and infection prevention aspects in the field of ASCT/HDCT.

Consistent with previous reports [1, 2, 6], contamination of HPCPs was a rare event (0.6%) in our study cohort and typically due to skin contaminants [1, 6]. The higher rate of contamination in BM cultures compared to cultures of PBPCs found in some earlier reports [2, 5, 10, 11] may explain the low rates of contamination in our cohort, where autologous BM harvesting was infrequent (reflecting current practice in most hematopoietic transplant centers). Interestingly, most CoNS were oxacillin-sensitive which is peculiar given that, more than 90% of CoNS cultured in Switzerland are oxacillin-resistant, according to the Swiss Center for Antibiotic Resistance. This may be due to the absence of concomitant antibiotic therapy (and accordingly, absence of previous selection of resistant isolates) at the time of sampling in our population.

The practice of infusing contaminated graft cells in patients with subsequent transient immunodeficiency may raise concerns among clinicians. However, according to the literature, infectious complications immediately following the infusion of autologous or allogeneic contaminated grafts are uncommon [2, 3, 6-9], and major adverse outcomes have rarely been reported [1]. This may either be due to the low pathogenicity of contaminants or the use of standard antimicrobial prophylaxis. The process of cryopreservation may play a certain protective role but does not guarantee sterility later on in the process, i.e. at thawing and upon

infusion. Finally, the empiric broad spectrum therapy in case of subsequent febrile neutropenia is often adequate for treating a usually low virulence contaminant.

When it comes to dealing with contaminated graft, different strategies have been described, ranging from preemptive therapy for each recipient to the administration of appropriate antibiotic therapy only when febrile neutropenia develops or a bacteremia is suspected (Table 2 summarizes the most relevant reports published on this subject since the year 2000). At our center, a specific preemptive therapy was initiated on an individual basis in six patients only. Importantly, there were no relevant adverse outcomes in any of the recipients of contaminated grafts, such as fever at the time of infusion of HPCPs or subsequent bacteremia with the same pathogen. Moreover, the standard empirical broad – spectrum antibiotic therapy started for neutropenic fever was - in all cases except one - adequate for the previously cultured contaminant. Of note, about three-quarters of recipients were on antimicrobial prophylaxis with TMP-SMX, currently a standard at our institution, which may have prevented subsequent BSI due to CoNS and *Bacillus* spp..

Given the rarity of significant consequences despite the absence of a preemptive therapy, it is questionable whether routine cultures are needed at all in this scenario and if they are cost effective. The two studies [10, 12] that addressed this issue found, that a routine surveillance generated an estimated yearly cost of $2,830,500 to $3,774,000 (in calendar year 2005) in the United States and was associated with an expense of $95,000 (1998) per bacteremia prevented. In our study, the overall cost for the 3935 cultures was approximately $260’000. Given the absence of major outcomes, a cost effectiveness analysis was not possible.

Our study is limited by its retrospective and single-center design, and our results may not be representative for and reproducible in other centers. Moreover, given that allogenic stem cells transplants are conducted in other medical centers across the country, our data on autologous stem cell transplants and our conclusions are restricted to this specific setting. Lastly, we were unable to identify any process step suspicious to entail HPCP contamination.

193 However, the large number of cultures reviewed is representative and our results allow concluding that
194 microbial contamination of autologous stem cell products is a rare event affecting only 0.6% of all products in
195 our study. Bacterial contamination of autologous HSPCs is mainly caused by low virulence skin contaminants,
196 and without major adverse outcomes. Contaminated grafts can safely be infused and preemptive antibiotic
197 therapy is not needed on a routine basis.

198

Bibliography

1. Klein, M.A., et al., *Microbial contamination of hematopoietic stem cell products: incidence and clinical sequelae.* Biol Blood Marrow Transplant, 2006. **12**(11): p. 1142-9.
2. Padley, D.J., A.B. Dietz, and D.A. Gastineau, *Sterility testing of hematopoietic progenitor cell products: a single-institution series of culture-positive rates and successful infusion of culture-positive products.* Transfusion, 2007. **47**(4): p. 636-43.
3. Dal, M.S., et al., *Does microbial contamination influence the success of the hematopoietic cell transplantation outcomes?* Transfus Apher Sci, 2016. **55**(1): p. 125-8.
4. Schwella, N., et al., *Bacterial contamination of autologous bone marrow: reinfusion of culture-positive grafts does not result in clinical sequelae during the posttransplantation course.* Vox Sang, 1998. **74**(2): p. 88-94.
5. Kozlowska-Skrzypczak, M., et al., *Microbial contamination of peripheral blood and bone marrow hematopoietic cell products and environmental contamination in a stem cell bank: a single-center report.* Transplant Proc, 2014. **46**(8): p. 2873-6.
6. Patah, P.A., et al., *Microbial contamination of hematopoietic progenitor cell products: clinical outcome.* Bone Marrow Transplant, 2007. **40**(4): p. 365-8.
7. Almeida, I.D., et al., *Autologous transplant: microbial contamination of hematopoietic stem cell products.* Braz J Infect Dis, 2012. **16**(4): p. 345-50.
8. Namdaroglu, S., et al., *Microbial contamination of hematopoietic progenitor cell products.* Transfus Apher Sci, 2013. **48**(3): p. 403-6.
9. Webb, I.J., et al., *Sources and sequelae of bacterial contamination of hematopoietic stem cell components: implications for the safety of hematotherapy and graft engineering.* Transfusion, 1996. **36**(9): p. 782-8.
10. Kamble, R., et al., *Microbial contamination of hematopoietic progenitor cell grafts-incidence, clinical outcome, and cost-effectiveness: an analysis of 735 grafts.* Transfusion, 2005. **45**(6): p. 874-8.
11. Jansen, J., et al., *Transplantation of hematopoietic stem cells from the peripheral blood.* J Cell Mol Med, 2005. **9**(1): p. 37-50.
12. Nasser, R.M., et al., *Routine cultures of bone marrow and peripheral stem cell harvests: clinical impact, cost analysis, and review.* Clin Infect Dis, 1998. **27**(4): p. 886-8.

Table 1: Cultures results of stem cells products and clinical characteristics of recipients of contaminated grafts between January 1, 2002 and December 31, 2019

Contamination	**N**	**%**
HPCPs[1] cultured	3,935	
HPCPs contaminated	25	0.6
Stage of detected contamination		
During apheresis	15	60
Before cryopreservation / at thawing[2]	10	40
Contaminant microorganisms	**N**	**%**
CoNS[3]	23	68
Streptococci	3	9
Bacillus spp.	2	6
Cutibacterium acnes	1	3
S. aureus	1	3
Gram-negative bacteria[4]	4	12
Baseline characteristics of the 22 recipients of contaminated grafts	**N**	**%**
Age (years, mean, range)	51	2-70
Female	7	32
Hematologic neoplasia	18	
Multiple myeloma	9	41
Lymphoma	7	32
Acute myeloid leukemia	2	9
Solid neoplasia	4	18
Germ line tumor	2	9
Prophylaxis with TMP-SMX[5]	16	73
Antibiotic therapy for other reasons at the time of HPCP infusion	2	9
Management of auto-graft contamination	**N**	**%**
Antibiotic preemptive therapy for HPCP contamination	6	27
Number of patients with blood cultures taken for febrile episodes after infusion	21	95
same microorganism detected	0	0
other microorganism detected	11	52
negative blood culture	10	48
Antibiotic therapy after infusion of contaminated HPCP for neutropenic fever		
already on preemptive therapy	6	27
adequate for contaminant	14	64
not adequate for contaminant	1	5
None (no neutropenic fever)	1	5
Outcome of the 22 patients	**N**	**%**
Hospital stay (median, days)	23	
In-hospital mortality	1	5
Long term follow up (median, weeks)	129	
Remission	17	77
Relapse	2	9
Death	3	13

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5 [2] One HPCP was positive before cryopreservation and at thawing, the others only at one stage during the
6 manufacturing process

7 [3] Coagulase negative staphylococci. 20 /23 (87%) were oxacillin susceptible, and 14 out of 14 (100%) with
8 available sensibility assays were trimethoprim-sulfamethoxazole susceptible.

9 [4] Gram-negative bacteria included: *Acinetobacter* spp. *Veillonella* spp. and *Ralstonia spp.* and not identified
10 Gram-negative bacilli.

11 [5] Trimethoprim-sulfamethoxazole. One additional recipient on atovaquone.

Table 2: Selection and summary of the most relevant reports on management of contaminated hematopoietic stem and progenitor cell products published since 2000

Studies	PMID	HPCPs cultured, N	HPCPs contaminated N, (%)	Antimicrobial prophylaxis	Antibiotic preventive strategy and N of recipients receiving preemptive antibiotic therapy	N of graft-related BSI / N of recipients
Kamble et al 2005	15934984	735	33 (4)	quinolone azole	based on attending physician preferences 4/26	0/26
Kelly et al. 2006	16785868	1502	15 (1)	TMP-SMX	based on attending physician preferences 5/13	1/13
Klein et al. 2006	17085307	2935	36 (1)	not specified	all recipients of contaminated HPCPs received antibiotic therapy one day before transplantation.	2*/35
Padley et al. 2007	17381622	7233	119 (2)	not specified	not specified 23/69	1/69
Patah et al. 2007	17572714	3078	37 (1)	quinolone, acyclovir, azole	all recipients of contaminated HPCPs received appropriate antibiotics, started before infusion	0/37
Almeida et al 2011	22846122	837	36 (4)	not specified	all recipients of contaminated HPCPs received appropriate antibiotics received pre- and post-infusion antibiotic therapy, established based on the isolated microorganism and susceptibility testing	0/22
Donmez et al. 2012	21981571	491	46 (9)	quinolone, azole	not specified 2/20	4/20
Namdaroglu et al 2013	23664302	1630	103 (6)	TMP-SMX, quinolone, azole	appropriate antibiotic therapy at febrile neutropenia or any sign of BSI	0/46
Dal et al 2016	27184293	1552	18 (1)	TMP-SMX, quinolone, azole acyclovir	appropriate antibiotic therapy at febrile neutropenia or any sign of BSI	2/9

*One recipient died because of sepsis due to *Pseudomonas cepacia*